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C. J. Peters, et al

Army Medical Research Institute of
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HEMORRHAGIC FEVER IN COCHABAMBA, BOLIVIA, 1971

C. J. PETERS,¹ R. W. KUEHNE,² R. R. MERCADO,³ R. H. LE BOW,⁴ R. O. SPERTZEL² AND P. A. WEBB¹

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Peters, C. J., R. W. Kuehne (Animal Assessment Div. U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md. 21701), R. R. Mercado, R. H. Le Bow, R. O. Spertzel and P. A. Webb. Hemorrhagic fever in Cochabamba, Bolivia, 1971. *Am J Epidemiol* 99:425-433, 1974.—Between January and March 1971, an unusual outbreak of hemorrhagic fever occurred in Cochabamba, Bolivia. The index case was apparently infected elsewhere, but became ill in Cochabamba, where hemorrhagic fever had never been reported. Four secondary cases occurred among hospital contacts of the index case, and a pathologist was infected after cutting himself during an autopsy of one of the secondary cases. Clinical manifestations were typical of Bolivian hemorrhagic fever, except that jaundice occurred in three cases. An arenavirus of the Tacaribe complex was isolated from three cases. One isolate was studied in detail and shown to be closely related antigenically to the prototype strains of Machupo virus.

arenavirus; hemorrhagic fever; jaundice; etiology; Machupo virus

INTRODUCTION

The arenavirus group is composed of 10 morphologically similar viruses having extensive immunologic relationships (1, 2). These agents are primarily rodent viruses and most are maintained in rodent popula-

tions, at least in part, by a mechanism of prolonged viremia and virus shedding. Four of the viruses (Lassa, lymphocytic choriomeningitis, Junin and Machupo) are known to cause disease in man. It is thought that lymphocytic choriomeningitis, Junin, and Machupo viruses infect man primarily through direct contact with the infected natural host or its excreta. The maintenance and communicability of Lassa virus are not well understood; however, the recognition of an explosive hospital epidemic establishes its potential for direct person-to-person transmission (3, 4).

During February and March 1971, fatal cases of a disease suspected of being Bolivian hemorrhagic fever (BHF) occurred in Cochabamba, Bolivia. This outbreak was investigated by local authorities in Cochabamba, the Ministry of Health of Bolivia, a representative of the Pan American Health Organization, and a physician from the Middle America Research Unit (MARU). We were concerned that this

Abbreviations: BHF, Bolivian hemorrhagic fever; CF, complement fixation; IC, intracranial; IFAT, indirect fluorescent antibody test; IP, intraperitoneal; MARU, Middle America Research Unit; PFU, plaque-forming units; USAMRIID, U.S. Army Medical Research Institute of Infectious Diseases.

¹Middle America Research Unit, Box 2011, Balboa Heights, Canal Zone.

²U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701. (Reprint requests to Mr. Kuehne, Animal Assessment Division.)

³Ministry of Health, La Paz, Bolivia.

⁴Peace Corps physician, U.S. Public Health Service.

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outbreak might not be classical BHF due to Machupo virus infection because: 1) secondary cases occurred among hospital personnel and contacts; 2) BHF had never occurred in the highlands of Bolivia where the rodent reservoir of Machupo virus, *Calomys callosus*, is not present; 3) some of the patients were icteric, a rare occurrence in BHF; and 4) a serum specimen from the lone survivor obtained 30 days after onset of illness contained little or no neutralizing antibody to Machupo virus. For these reasons, samples from acutely ill patients were studied in collaboration with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Frederick, Maryland, where appropriate safety facilities were available.

METHODS

Description of laboratory facilities. The special containment laboratories used in this study have been described elsewhere (5). Two separate, gas-tight cabinet systems were used so that materials from the outbreak, as well as a prototype Machupo virus strain could be evaluated concurrently without danger of cross-contamination. We adhered to the *Guide for Laboratory Animal Facilities and Care* of the Institute of Laboratory Animal Resources National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

Processing of specimens. Throat swabs were agitated in 1 ml of holding medium composed of veal infusion broth (Difco) containing 5 mg bovine serum albumin (Fraction V, Armour), 2.5 μ g streptomycin, and 500 units penicillin G per ml. All specimens intended for virus isolation were frozen as soon as possible and stored continuously in liquid nitrogen or a mechanical freezer at -60 C. Human blood for serologic testing was collected in Vacutainers (Becton-Dickinson, Rutherford, N.J.) and held at 4 C for no longer than 24 hours.

The serum was separated from the clot by centrifugation and stored at -20 C.

Preparation of virus suspensions. Organs or whole blood were ground in a mortar with pestle and sand; four volumes of phosphate buffered saline (pH 7.4) containing 25 per cent normal rabbit serum (previously heated at 56 C for 30 minutes), 100 units/ml of penicillin G and 100 μ g/ml of streptomycin were added. The suspension was clarified by centrifugation in a table-top centrifuge at 2500 rpm for 10 minutes. Additional dilutions were made with cold phosphate buffered saline containing 10 per cent heated normal rabbit serum and antibiotics.

Animal and tissue culture inoculation. Animals for inoculation were obtained from these sources: Swiss Webster strain adult mice and pregnant dams from Charles River Breeding Laboratories, Wilmington, Mass.; Golden Syrian adult hamsters from Lakeview Hamster Colony, Newfield, N.J.; pregnant hamsters from Zucca's Hamstery, Vineland, N.J.; Hartley guinea pigs from West Jersey Biological Supply, Wenonah, N.J., or the Fort Detrick Animal Farm, Frederick, Md.

Suckling mice (three days of age or less) and suckling hamsters (five days of age or less) were inoculated intracranially (IC) and/or intraperitoneally (IP) with approximately 0.015 ml of virus suspension, using blunt-tipped, 26-gauge needles (Becton-Dickinson Co., Rutherford, N.J.). Suckling guinea pigs (five days of age or less) were inoculated either IC or IP with 0.1 ml of virus suspension. Adult animals received 0.1 ml of virus suspension by the IP route.

Vero, WI-38 and BHK-21 cell cultures were provided by the Virology Division, USAMRIID. Primary monkey kidney, human embryo kidney and MA-111 (continuous rabbit kidney cell line) were purchased from Microbiological Associates, Inc., Bethesda, Md. Plaque titrations were performed in Vero cell monolayers by a previously described technique (6), modi-

fied by using an overlay composed of Eagle's basal medium with Earle's salts, 2 per cent fetal calf serum, 1 per cent Noble agar, and 20 mM HEPES buffer.

Serologic tests. Complement-fixation (CF) tests for serum antibody were performed by a microtiter technique (7). The same test system was also used to identify crude antigens, which were prepared in the same manner as virus pools except that rabbit serum was not added to the diluent. Attempts to perform accurate plaque-reduction neutralization tests at USAMRIID were frustrated by our inability to consistently maintain Vero cell monolayers under agar. Subsequently, the neutralization tests were repeated at MARU, using reagents prepared at USAMRIID. Direct (8) and indirect (8) fluorescent antibody tests were performed with acetone-fixed viral antigens.

Virus strains and antisera. Reference reagents were obtained from MARU and are essentially as described in recent publications (9, 10). The prototype virus used for comparison was the second or third suckling hamster brain passage of the Carvallo strain isolated from the spleen of a fatal human case of BHF in San Joaquin, Bolivia.

DESCRIPTION OF THE OUTBREAK

Antecedent events and the course of the epidemic were reconstructed from hospital records and interviews in Cochabamba, Fortaleza and Tarija. Conflicting or absent data prevented precise conclusions, but the most probable course of events is summarized in figure 1.

Case 1: CM, a 20-year-old, single, nursing student living in Cochabamba, returned to her home village of Fortaleza in the Province of Beni, about December 6, 1970 (figure 2). She may have traveled in the vicinity of Fortaleza, but there was no indication that a known BHF endemic area was visited. She left Fortaleza to travel to Trinidad, Bolivia, about January 12, 1971.

Between January 12 and 15, she became ill and on January 15 returned to Cochabamba where she was soon admitted to the Seton Hospital. She died on January 28.

Case 2: JB, a 33-year-old domestic servant, assisted in the case of her sick niece (case 1) on January 25 and 26. She became ill on February 6 and was admitted to the Viedma Hospital on February 12, where she died on February 23.

Case 3: RM, father of case 1, traveled to Cochabamba from his ranch in Fortaleza because of his daughter's illness, arriving on January 26. He was in intimate contact with her, kissing and embracing her during the last two days of her life. Case 3 returned to the Beni and probably became ill in Fortaleza about February 8. He traveled to the hospital in Trinidad and died there on February 16. We have little data concerning the course of his illness, but the physicians in Trinidad believed that he had died with hemorrhagic fever. Only this case and case 1 had been in the Beni prior to their illness.

Case 4: MS, a 28-year-old nursing instructor who supervised nursing care of case 1 on January 23-25 and was intimately involved in her care during the final days of life (January 26-28), became ill about February 7. She entered Seton Hospital on February 12 and died on February 19.

Case 5: FP, a 24-year-old nurse from Tarija, was in Cochabamba between January 16 and 27 attending a training course in hospital administration at Seton Hospital; her only contact with patients was on January 27, when she assisted case 4 with the bedside care of case 1. Case 5 had symptoms of gastroenteritis beginning on January 25, but appeared to be well by February 2. However, on February 5 she complained of malaise and by February 8 was definitely ill. She was hospitalized in Tarija on February 11 and recovered after a severe illness.

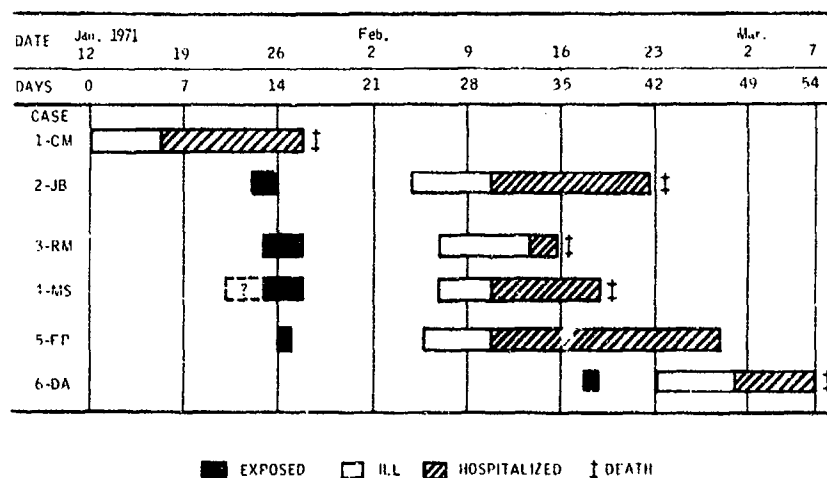


FIGURE 1. Temporal sequence of cases of Bolivian hemorrhagic fever, Cochabamba, 1971.

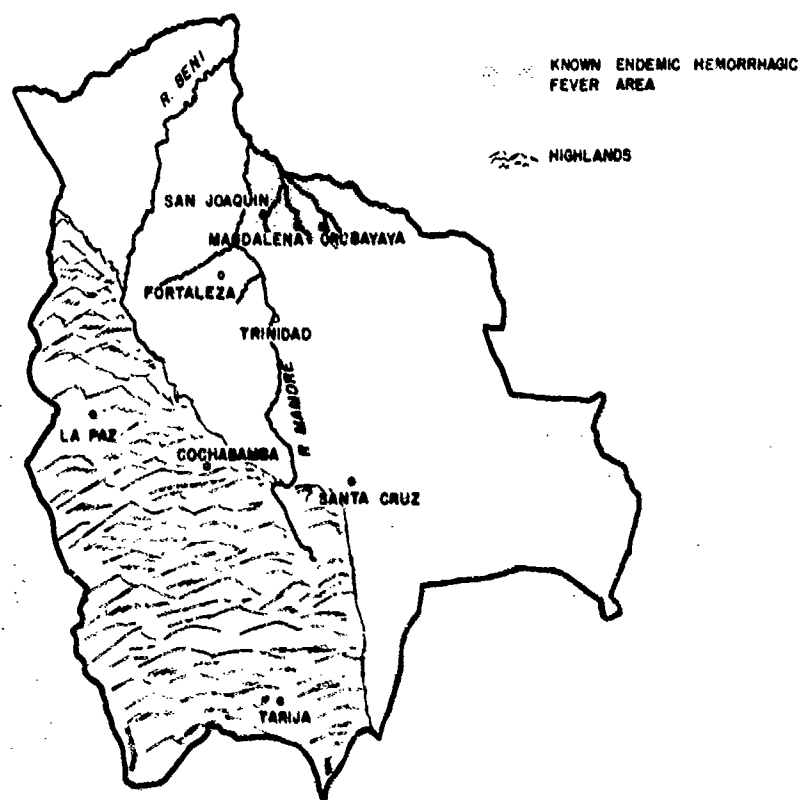


FIGURE 2. Map of Bolivia showing known endemic area of Bolivian hemorrhagic fever.

Case 6: DA, a 32-year-old pathologist in Seton Hospital, received a superficial scalpel cut on the thumb while performing an autopsy on case 4 approximately three hours after her death. He immediately

plunged his hand into formalin, scrubbed the cut, and continued the autopsy with fresh gloves. Four days later, on February 23, he had a fever of 38°C and two days later was obviously ill with malaise and

high fever. He was hospitalized at Seton Hospital on March 2 and died on March 7. This was the only infection in which parenteral exposure occurred.

Nine other persons lived in the same households as cases 2, 4, 5 and 6, and all of the patients had casual contacts with many other people during the incubation period and early stages of illness. Initially, most patients were suspected of having typhoid fever or gram-negative sepsis, so that isolation procedures were not put into effect until February 18. Nursing personnel and other contacts were exposed to bloody secretions, urine, vomitus, and bronchopulmonary aerosols. Cases 1 and 4 had private rooms at Seton Hospital, where at least 32 nursing or other paramedical personnel and seven physicians were involved in their case. Case 2 was nursed in an open ward at Viedma Hospital and was attended by 11 nurses and six doctors. Eight clinical laboratory technicians were known to have performed analyses on blood specimens taken from the patients. Surgical masks, gowns and gloves were worn when autopsies were performed on cases 2 and 4, but two persons received needle punctures and one person (case 6) was cut. After the onset of illness in case 6, hemorrhagic fever was suspected and he was cared for in Seton Hospital with mask, cap, gown and glove precautions. Three nurses, six physicians, and three clinical laboratory technicians were exposed. Case 6 was autopsied by two persons wearing mine respirators and heavy rubber gloves. No further cases were reported in spite of intense publicity and a state of near panic in the city.

CLINICAL MANIFESTATIONS

The clinical picture of the five cases for which we have data was very similar. Four to seven days before hospitalization, the subjects experienced fever, chills, myalgia, malaise and weakness. Anorexia, vomiting, abdominal tenderness and, in some cases, diarrhea developed. Four patients had con-

junctival and pharyngeal injection. During the first two or three days of hospitalization, all patients continued to be nauseated and febrile (38-40 C). Laboratory findings consisted of leukopenia, proteinuria, pyuria, and sometimes hematuria. Abnormalities of hemostasis developed: gingival hemorrhages, epistaxis, metorrhagia, ecchymoses around needle punctures, purpura, melena, hematuria.

During the later phase of the illness, patients became obtunded and hypotensive. Three cases became obviously jaundiced on the tenth to twelfth days of illness, and this was supported by the icteric serum samples observed in the laboratory. In particular, case 4 had 6.8 mg/100 ml total and 4.2 mg/100 ml direct bilirubin on the eleventh day of illness. There were tremors of the tongue and upper extremities, myoclonic movements and, in some cases, focal or generalized convulsions. In four cases, severe hypotension and coma were followed by death after six to 11 days of hospitalization. One subject (case 5) improved after 11 days of hospitalization and eventually recovered. All cases were treated with combinations of antibiotics, steroids and intravenous fluids.

Postmortem examinations were performed on cases 2, 4 and 6. Histopathologic findings in all three cases were typical of BHF (11). Scattered foci of hepatocellular necrosis and occasional acidophilic bodies (12) were observed in liver sections but these changes were no more extensive than those usually found in fatal BHF cases. Thus the jaundice observed in cases 4 and 6 remains unexplained. Additional findings included severe necrotizing bronchopneumonia and a septic vena cava thrombosis in case 3, early bronchopneumonia in case 4, and several areas of lobular pulmonary consolidation in case 6.

RESULTS

Virus isolation. Virus was recovered from the pre-mortem bloods of three cases, from

the throat swab of one patient and from liver (case 4) and spleen (case 6) obtained at autopsy. Titers obtained are shown in table 1. Although significant quantities of virus were present in the throat swab from case 6, there was blood from gingival hemorrhages in the specimen. We were not able to isolate virus from two serial urine specimens (case 6) obtained on days 10 and 12 after the onset of illness. Suckling hamsters were more susceptible to illness and death than suckling mice, but virus could be isolated from either animal. Spleen homogenate from case 6 inoculated IP into adult hamsters, mice and guinea pigs, did not induce illness or death but surviving animals developed CF antibody to Machupo virus when bled 30 days post-inoculation.

Cytopathic effects were observed only in WI-38 and MA-111 cell cultures. Direct fluorescent antibody tests showed that at least 50 per cent of the Vero cells grown on Leighton coverslips contained large amounts of viral antigen as early as five days after inoculation.

Properties of the isolate. The isolate obtained from the spleen of case 6 (DA) was chosen as the prototype of the Cochabamba outbreak. A suckling hamster brain pool of the DA strain in the second passage level was used in all comparative studies with the prototype Carvallo strain of Machupo virus. The DA pool contained 6.6×10^5 plaque-forming units (PFU) per

ml (range of 45 tests done on five days: 5.1×10^4 to 1.3×10^5) and the Carvallo pool, 1.4×10^5 PFU/ml (range of 49 tests done on six days: 6.9×10^4 to 3.1×10^5).

Infection of WI-38 cells with the two strains produced focal areas of ballooning and detachment of cells within 10-14 days that progressed to involvement of the entire cell sheet within 15-21 days. Although MA-111 cells became rounded and granular in the original isolation attempts, we were unable to reproduce this finding with passage material from the DA isolate, or with the Machupo Carvallo strain. Passage material from both strains grew in Vero cells as determined by detection of viral antigen with fluorescent technique, but no consistent changes in cell morphology were seen. The DA strain from Cochabamba and the prototype Machupo virus from San Joaquin formed similar plaques under agar in Vero cells.

Plaque reduction neutralization of hyperimmune hamster sera to the DA and Carvallo strains is shown in table 2. DA hyperimmune hamster sera had a fourfold to eightfold lower neutralization titer against Carvallo than against its homologous DA strain. In contrast, a Carvallo immune serum had similar titers to both agents.

Suckling mice and suckling hamsters inoculated IC suffered characteristic illness, consisting of growth retardation, tremors and convulsions, usually followed by death between days 9 and 16. Titrations of virus pools in suckling hamsters, although more variable, usually gave endpoints of the same order of magnitude as those of plaque titrations for both strains.

The Carvallo strain of Machupo virus was more virulent for guinea pigs than DA virus. Four of six adult animals inoculated IP with the former agent died whereas none of six guinea pigs infected with a similar amount of DA virus succumbed. Suckling guinea pigs less than five days old were infected by IP and IC routes to comparable graded doses of each virus. Mortality was

TABLE 1
Detection of virus in cases of hemorrhagic fever

Case	Specimen	Days of illness	PFU/ml	SIITU, 2 ml
<i>Pre-mortem</i>				
6	Throat swab	10	6.0×10^5	2.0×10^5
	Blood	10	2.0×10^5	8.0×10^5
	Blood	11	1.0×10^5	
4	Blood	12	1.6×10^5	
2	Blood	13	1.0×10^5	
<i>Post-mortem</i>				
4	Liver	12		2.0×10^5
6	Spleen	12	2.0×10^5	2.0×10^5

* Plaque forming units.

† Median suckling hamster infective doses.

TABLE 2
Relationships between DA and Carvallo strains of Machupo virus in neutralization test

Anti-serum*	Immunizing strain	Injections	Reciprocal titer to indicated virus	
			DA	Carvallo
1	DA	3	32†	8
2	DA	3	32	4
3	Carvallo	3	32	32
4	Carvallo	6	256	512

* All antisera prepared in adult hamsters.

† Titer expressed as last serum dilution inhibiting 80% of 50-100 virus plaques.

independent of virus dose when given IC. Carvallo virus killed 49 of 56 animals as compared to five of 29 DA-infected guinea pigs ($p < .001$). Although there was a direct dose-response effect when the agents were administered IP, the mortality at each virus dose was greater for Carvallo than DA virus (table 3). Death of guinea pigs usually occurred between 18 to 23 days after inoculation and was preceded by a period of weight loss and decreased activity.

Serologic studies. Convalescent serum from case 5, the only survivor of the outbreak, was tested by neutralization and CF to various arenaviruses other than Machupo. Although a titer of 1:64 was obtained by CF using Junin antigen, neutralization tests with this virus and Tacaribe, Amapari, Parana and Latino viruses were negative. Serum neutralizing antibody titers of this patient to both strains of Machupo virus are shown in table 4. An eightfold higher titer was obtained when the seven-week convalescent serum was tested against the DA strain than the Carvallo strain. Unfortunately, only a small volume of serum had been obtained four weeks post-infection and none was available for testing against the DA strain. By comparison, seven- to eight-week convalescent sera from the 1963-1964 San Joaquin BHF outbreak had similar titers to both Machupo and DA viruses.

From March 7 to 13, 1971, we obtained

serum samples from 125 hospital and household contacts of the hemorrhagic fever cases. Two months later, 61 persons were bled for a second time. All sera were examined for CF antibodies to Machupo virus. Of these, 20 had anticomplementary activity or equivocal reactions at 1:2 or 1:4 dilutions; these were tested by neutralization and indirect fluorescent antibody tests (IFAT) and found to be negative. Paired sera from one subject, a 42-year-old nurse, had low levels of CF (1:2) and IFAT (1:4) antibodies, but no detectable neutralizing antibody. She had never been in the Beni Province, had never knowingly cared for a patient with hemorrhagic fever and had no previous illness suggestive of hemorrhagic fever.

DISCUSSION

Although two other cases of direct human transmission of Machupo virus are on record (15, 16), this outbreak represents the first instance in which this mode of virus spread was of primary importance. Thus, as in the case of related arenavirus Lassa, the possibility of occurrence of "mysterious" geographically exotic outbreaks of BHF is now real.

Despite the fact that no direct statistical comparison is possible between these patients and those previously studied in the Beni Province, it appears that the basis for direct virus transmission was the presence of large amounts of virus in blood and

TABLE 3
Lethality of two strains of Machupo virus for suckling guinea pigs infected by intraperitoneal route

Dose	Carvallo	Dose	DA
1000	21/25†	7000	7/21
100	11/17	700	2/12
10	11/11	70	3/15
1	6/18	7	1/22

* Plaque forming units.

† Mortality curve Carvallo strain significantly greater ($p < .005$) than that for DA strain by Hanky-Myers test (14).

‡ Number deaths/number inoculated.

TABLE 4

Neutralization of two Machupo virus strains by convalescent Bolivian hemorrhagic fever human sera

Case	Locality	Weeks post-illness	Reciprocal titer to indicated virus	
			DA	Carvallo
FP	Cochabamba	4	NT*	4†
	Cochabamba	7	32	4
KJ	San Joaquin	8	8	8
RL	San Joaquin	7	32	16
GL	San Joaquin	7	64	32

* Not tested, serum exhausted.

† Titer expressed as last serum dilution inhibiting $\geq 80\%$ of 50-100 virus plaques.

secretions. In San Joaquin during 1963-1964, virus was recovered from blood and throat secretions in only about one in five attempts during serial sampling of patients with confirmed BHF, and the quantities of Machupo virus present were minimal (17).

We cannot decide whether this outbreak was caused by a Machupo virus variant having distinctive biologic properties or whether the observed icterus, hitherto distinctly unusual in BHF (18, 19), the high rate of clinically significant secondary bacterial infection, the striking mortality and the direct human virus transmission were somehow related to the fact that this epidemic occurred in persons "adapted" to mild physiologic stress (Cochabamba is nearly 2700 meters above sea level). The latter possibility might be tested experimentally by infecting nonhuman primates maintained in altitude chambers. Evidence for the first alternative consists of the reduced virulence of the DA virus for guinea pigs as compared to a low-passage strain of Machupo virus and the fact that a minor but reproducible antigenic difference between these strains was found in a human serum and in two distinct antisera prepared against the DA strain.

Another point potentially in favor of a distinct Machupo variant was the fact that the index case apparently contracted her infection near Fortaleza, a town in Beni Province outside the previously docu-

mented enzootic area of BHF. Intensive trapping of this ranch in 1971 and 1972 yielded 30 rodents, none of which was *Calomys callosus*, the only known natural reservoir of Machupo virus (19). Further work is needed to determine whether other vertebrates are involved in virus transmission in this area.

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